

## Articles

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# Hepatitis A Vaccination

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The safety and immunogenicity of an inactivated hepatitis A virus vaccine were assessed in 101 healthy adults. Seronegative persons with normal serum aminotransferase levels were grouped according to age: Group 1 ( $n = 24$ ) and group 3 ( $n = 22$ ) were between 18 and 40 years of age, and group 2 ( $n = 25$ ) and group 4 ( $n = 30$ ) were older than 40 years. Groups 1 and 2 received vaccine on a 0-, 1-, and 2-month schedule (schedule A), and groups 3 and 4 received the vaccine on a 0-, 1-, and 12-month schedule (schedule B). Of the 101 vaccinated subjects, 98 (97%) seroconverted with antibody titers to hepatitis A virus of  $\geq 20$  IU per liter after the first dose, and all subjects seroconverted after the second dose. The geometric mean titers a month after the third vaccine dose were significantly greater ( $P < .03$ ) on both schedules for younger subjects (schedule A, 1,743 IU per liter, and schedule B, 7,882 IU per liter) than for older subjects (schedule A, 826 IU per liter, and schedule B, 4,279 IU per liter). Also, the differences in geometric mean titers a month after the third dose were significantly greater ( $P < .001$ ) for subjects in both age groups on schedule B (group 3, 7,882 IU per liter, and group 4, 4,279 IU per liter) than for those on schedule A (group 1, 1,743 IU per liter, and group 2, 826 IU per liter). The hepatitis A virus vaccine was well tolerated, with mild discomfort at the injection site being the main side effect. This vaccine is both safe and highly immunogenic.

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**H**epatitis A virus (HAV) infection continues to be a public health problem in the United States. In 1989, about 39,000 cases were reported to the Centers for Disease Control, and this viral infection accounts for as much as 50% of all cases of hepatitis that occur each year in this country.<sup>1</sup> In addition, large epidemics of acute hepatitis A continue to occur. In a recent study from Shanghai, China, more than 300,000 cases of acute hepatitis A resulted from the ingestion of uncooked clams contaminated with HAV.<sup>2</sup>

Several studies have been done recently on the development and testing of HAV vaccines.<sup>3-7</sup> Both attenuated and killed preparations of HAV vaccines have been used, and the preliminary reports on their safety and immunogenicity appear promising. In addition, in a recent report, a single dose of inactivated HAV vaccine was used and showed protective efficacy against clinical hepatitis A in an upstate New York community that was endemic for this disease.<sup>8</sup>

We report herein our experience with a formalin-inactivated HAV vaccine that consisted of the HM175 strain propagated in MRC5 cells. In this study, using a dose of HAV vaccine that had been previously shown to induce a maximal antibody response,<sup>3</sup> we administered vaccine in two different dosing schedules. The immunologic response to the HAV vaccine in two different age groups was assessed.

## Subjects and Methods

Staff members from the Huntington Memorial Hospital (Pasadena, California) volunteered for HAV vaccine administration. The inclusion criteria were good health, age 18 years or older, serum tests negative for anti-HAV, normal serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, and a signed informed consent. Persons who were excluded had received blood, blood products, or immune globulin during the six months before the study, expected to receive blood or blood products or immunosuppressive therapy while enrolled in the study, were enrolled in other vaccine trials, or were lactating or pregnant.

Within seven days of entry into the study, a history was obtained and a physical examination was done on all volunteer subjects. Blood specimens were collected for an initial anti-HAV titer and for tests of liver function. Eligible subjects were randomly assigned to either of two dosing schedules: schedule A: 720 enzyme-linked immunosorbent assay (ELISA) units of the HAV vaccine was administered at months 0, 1, and 2; schedule B: 720 ELISA units was administered at months 0, 1, and 12. Four groups of subjects were studied: subjects 18 to 40 years of age received the HAV vaccine according to either schedule A (group 1) or B (group 3), and subjects older than 40 years received the HAV vaccine according to either schedule A (group 2) or B (group 4).

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## ABBREVIATIONS USED IN TEXT

ALT = alanine aminotransferase  
 AST = aspartate aminotransferase  
 ELISA = enzyme-linked immunosorbent assay  
 HAV = hepatitis A virus  
 HBsAg = hepatitis B surface antigen

At day 0 (month 0), subjects on both schedules were administered HAV vaccine intramuscularly into a deltoid muscle and were observed for 15 minutes for any adverse reaction. A diary card was provided to record any adverse effects occurring within three days after vaccination.

At month 1 ( $30 \pm 7$  days after the first dose), the diary cards were retrieved, blood specimens were obtained to measure serum ALT and AST levels and anti-HAV titers, and the second dose of HAV vaccine was administered. The subjects were observed for 15 minutes for any adverse effects and again were provided with a diary card to record any adverse reaction occurring within three days after vaccination.

Subjects allocated to schedule A returned at month 2 ( $60 \pm 7$  days after the first dose) for the third dose of HAV vaccine, and the same procedure was followed as at month 1. Additional blood specimens were drawn to measure anti-HAV titers and serum ALT and AST levels at months 3, 12, and 13 (all  $\pm 7$  days).

Subjects allocated to schedule B returned at months 2, 3, 12, and 13 (all  $\pm 7$  days) to have blood specimens taken for measuring anti-HAV titers and ALT and AST levels. At month 12 ( $360 \pm 7$  days after the first dose), after the blood specimens were obtained, a third dose of HAV vaccine was administered. The subjects again were observed for 15 minutes for any adverse effects, and a diary card was provided to record adverse reactions occurring within three days after vaccination. Diary cards were retrieved at months 1, 2, and 13.

## Vaccine

The candidate inactivated hepatitis A vaccine (Havrix) used in this study was developed using the HM175 strain of HAV, which was originally derived from human stool and propagated in MRC5 cells according to the seed

lot principle.<sup>3</sup> The antigen content of this vaccine has been referenced to a standard by the ELISA and is expressed in ELISA units. This HAV vaccine meets the requirements of the World Health Organization for other inactivated vaccines, such as the poliovirus.<sup>6</sup> Each 1-ml dose contains HAV antigen, 720 ELISA units; aluminum hydroxide corresponding to 0.5 mg of aluminum as the adjuvant; and 5.0 mg of 2-phenoxyethanol added as a preservative. Other excipients include 0.05 mg of polysorbate 20, 3.0 mg of amino acid supplement, 1.15 mg of disodium phosphate, 0.20 mg of monopotassium phosphate, 9.0 mg of sodium chloride, and 0.23 mg of potassium chloride.

## Data Analysis

Initially a commercial test kit (HAVAB, Abbott Laboratories, North Chicago, Illinois) was used to determine the presence or absence of anti-HAV in screening blood specimens. The postvaccination serum specimens were sent to SmithKline Biologicals in Rixensart, Belgium, in a blinded manner for the determination of anti-HAV titers by ELISA,<sup>3</sup> and titers were reported in international units per liter. Seroconversion was defined as an anti-HAV titer of 20 IU per liter or higher. The geometric mean titers were calculated by adding together the log of each titer and then determining the anti-log of the mean of the logs. For analysis, the geometric mean titers were compared at several time points for the two age groups using a two-tailed Student's *t* test.

## Results

A total of 175 subjects were screened for anti-HAV and serum ALT and AST levels before entry into the study. Of these, 55 were either anti-HAV-positive or had elevated serum ALT or AST levels and were not eligible. Another 14 subjects did not complete the vaccination schedule; that is, they missed one or more vaccinations or missed scheduled appointments to have blood specimens taken and were not included in the final analysis. Another four had adverse reactions to the first dose of vaccination, and one woman became pregnant after two vaccinations, all of whom were withdrawn. The final analysis included 101 subjects who completed the entire series of vaccination and had anti-HAV titers done after each vaccination.

TABLE 1.—The Demographic Characteristics of 101 Recipients of Hepatitis A Virus Vaccine

Demographics	Schedule A		Schedule B	
	$\leq 40$ yr Group 1	$> 40$ yr Group 2	$\leq 40$ yr Group 3	$> 40$ yr Group 4
Mean age, yr*	$30 \pm 7$	$52 \pm 5$	$33 \pm 5$	$46 \pm 5$
Mean weight, kg (lb)*	$66.4 \pm 12.7$ ( $146 \pm 28$ )	$73.6 \pm 17.7$ ( $162 \pm 39$ )	$68.6 \pm 15.9$ ( $151 \pm 35$ )	$69.5 \pm 13.6$ ( $153 \pm 30$ )
Men, No.	6	9	5	6
Women, No.	18	16	17	24
Race, No.				
White	11	21	14	28
African American	4	2	3	1
Hispanic	5	1	2	0
Asian	4	1	3	1

\* $\pm$  Standard deviation.

The demographic characteristics for the 101 subjects are shown in Table 1. The average age of the vaccinees was 40 years  $\pm$  7 SD. The age, sex, and race for subjects in groups 1 through 4 are also shown.

Eleven subjects had transient increases in serum ALT or AST levels 1.5 times above normal (35 U per liter) on one (7 subjects) or more (4 subjects) occasions during the study. The last four subjects did not have antibodies to the hepatitis C virus when assayed by a second-generation test and were negative for the hepatitis B surface antigen (HBsAg). In all instances, the serum aminotransferase levels returned to normal when tested three months after the HAV vaccination.

### Adverse Effects

Transient discomfort at the injection site was reported after 11% of all vaccinations (Table 2). Gastrointestinal symptoms and headaches were infrequent. Most of the side effects were reported after the first dose of HAV vaccine. Injection site discomfort also was experienced after the second and third vaccinations but usually was not reported by the same vaccinees. Four subjects were withdrawn from the study because of an adverse reaction after the first injection of HAV vaccine and were deemed to be unsuitable for further vaccination. These four cases are described in Table 3.

### Immunogenicity

Of the 101 subjects, 98 (97%) seroconverted after one dose of HAV vaccine. All subjects seroconverted after two doses of HAV vaccine. At month 2, a month after the second dose of vaccine, there were no significant differences in the geometric mean titers between the younger and older subjects (Table 4). The geometric mean titers a month after the third vaccine dose were significantly greater ( $P < .03$ ) on both schedules for younger than for older subjects. Also, the differences in geometric mean titers a month after the third dose were significantly greater ( $P < .001$ ) for subjects in both age groups on schedule B than for those on schedule A (see Table 4).

### Discussion

In this report, administering a formalin-inactivated hepatitis A vaccine to anti-HAV-negative hospital staff volunteers resulted in the production of antibodies to HAV in 100% of the persons vaccinated. When tested after the third dose of vaccine, subjects who received the 0-, 1-, and 12-month schedule had significantly higher titers of antibodies to HAV than those who received the 0-, 1-, and 2-month schedule. Also, our results using 720 ELISA units of inactivated hepatitis A vaccine at the 0-, 1-, and 12-month dosing schedule resulted in higher anti-HAV titers than those in a previous study using identical vaccine at varying doses administered at 0, 1, and 2 months.<sup>3</sup> Thus, our study shows that a time lag of longer than a month between the second and third dose of hepatitis A vaccine is required for higher antibody levels. For both of our vaccine dosing levels, younger subjects had higher HAV antibody levels than the older subjects. This

TABLE 2.—Adverse Effects Reported After the Hepatitis A Virus Vaccine

Symptom	Complaints After All Injections, %
Pain at injection site .....	11
Nausea, vomiting .....	3
Headache .....	1
Fatigue .....	1
Dizziness .....	1
Backache .....	1
Flulike symptoms .....	1
Wheezing .....	1

last finding is similar to that reported with the hepatitis B vaccine in which the geometric mean titers of HBsAg after vaccination were noted to be lower in the older subjects.<sup>9</sup>

This hepatitis A vaccine was well tolerated, with transient injection site discomfort reported as the main side effect. Four subjects who had adverse reactions that may have been related to vaccine injection refused further vaccinations (see Table 3). Although possible, wheezing and rash have not been reported previously with this vaccine.

TABLE 3.—Summary of 4 Persons With Adverse Reactions to the Initial Dose of Hepatitis A Virus Vaccine

Subject	Case Description
1 .....	A 56-year-old woman had wheezing after vaccination, which lasted 30 minutes; no treatment was required, and she recovered
2 .....	After the first dose of vaccine, a 20-year-old man had an achy jaw, upset stomach, flulike symptoms, and fatigue that lasted 2 days
3 .....	1 Day after vaccination, a 63-year-old woman had a flulike illness, malaise, and nausea that lasted 24 hours
4 .....	5 Days after vaccination, a 74-year-old man noted the onset of a papular rash, tender scalp, painful finger pads, malaise, and abdominal discomfort; he was given diphenhydramine hydrochloride and prednisone with immediate recovery

We noted mild serum ALT elevations in 11 subjects. When remeasured during the follow-up period, the serum ALT values all had returned to normal. In the four persons who had more than one abnormal ALT value, the HBsAg test and the test of antibodies to the hepatitis C virus measured by a second-generation test were negative. Other studies on the use of HAV vaccine in healthy populations also reported slight but transient elevations of serum transaminase levels.<sup>4,5</sup> If a live attenuated HAV vaccine is used, it is conceivable that infection of hepatocytes may take place, but in the case of an inactivated vaccine preparation, other causes of serum aminotransferase elevation must be considered. In our subjects, the reasons for the ALT and AST elevations were not explained.

In two previous reports in which inactivated HAV vaccines were used, neutralizing antibodies were detected in 86% to 100% of subjects.<sup>5,6</sup> When the commercial HAVAB radioimmunoassay was used, one study reported

TABLE 4.—Geometric Mean Titers of Antibodies to Hepatitis A Virus (HAV) in Recipients of Vaccine (given in IU/liter)

		Month After Initial Dose of Vaccine				
		1	2	3	12	13
Subjects, No.						
Schedule A						
≤40 yr .....	24	219*	446†‡	1,743§¶	806‡	839
>40 yr .....	25	190*	284†‡	826§¶	523‡	593
Schedule B						
≤40 yr .....	22	227*	590‡	514	351†	7,882§¶
>40 yr .....	30	269*	269‡	427	311†	4,279§¶
*Received second dose of HAV vaccine.		†Received third dose of HAV vaccine.		‡P < .20.	§P < .03.	¶P < .001.

\*Received second dose of HAV vaccine. †Received third dose of HAV vaccine. ‡P < .20. §P < .03. ¶P < .001.

only a 40% positivity, and none of the subjects had detectable anti-HAV immunoglobulin M by HAVAB-M.<sup>5</sup> In the other report, 31 of 32 (97%) had detectable anti-HAV by a radioimmunoassay technique that was different from HAVAB.<sup>6</sup> Two other studies using a single dose of attenuated live HAV vaccine showed that anti-HAV was detected in all subjects after vaccination, and neutralizing antibodies were also present in all of the vaccinees.<sup>4,7</sup> These preliminary reports indicate that both the live attenuated type and the killed type of HAV vaccines were immunogenic and well tolerated. In our study, the commercial HAVAB test was used as the initial screening test for anti-HAV. This test was different from the ELISA test that was done at SmithKline Biologicals in Belgium for detecting antibodies after HAV vaccination.<sup>3</sup> Although it is conceivable that some of the vaccinees may have had an initial positive titer by the ELISA test, none of them had an anamnestic response after the first dose of HAV vaccine, indicating that none of our subjects had had a previous exposure to the hepatitis A virus. Thus, the progressively rising antibody levels to HAV that were detected in our vaccinees were a result of the three HAV vaccine doses administered. In summary, the formalin-inactivated HAV vaccine that was used in this investigation

produced higher antibody titers in subjects younger than 40 years, and the dosing schedule of 0, 1, and 12 months resulted in higher antibody levels than the 0-, 1-, and 2-month schedule. The HAV vaccine will be important in preventing acute hepatitis A and will contribute toward the eradication of this ubiquitous viral illness.

#### REFERENCES

1. Shapiro CN, Show FE, Mandel EJ, Hadler SC: Epidemiology of Hepatitis A in the United States. In Hollinger FB, Lemon SM, Margolis H (Eds): *Viral Hepatitis and Liver Disease*. Baltimore, Md, Williams & Wilkins, 1991, pp 71-76
2. Yao GB: Clinical spectrum and natural history of viral hepatitis A in a Shanghai epidemic. In Hollinger FB, Lemon SM, Margolis H (Eds): *Viral Hepatitis and Liver Disease*. Baltimore, Md, Williams & Wilkins, 1991, pp 76-78
3. Andre FE: Development of a vaccine against hepatitis A. In Hollinger FB, Lemon SM, Margolis H (Eds): *Viral Hepatitis and Liver Disease*. Baltimore, Md, Williams & Wilkins, 1991, pp 85-88
4. Midhun K, Ellerbeck E, Gershman K, et al: Safety and immunogenicity of a live attenuated hepatitis A virus vaccine in seronegative volunteers. *J Infect Dis* 1991; 163:735-739
5. Sjogren MH, Hoke CH, Binn LN, et al: Immunogenicity of an inactivated hepatitis A vaccine. *Ann Intern Med* 1991; 114:470-471
6. Flehmig B, Heinricy U, Pfisterer M: Immunogenicity of a killed hepatitis A vaccine in seronegative volunteers. *Lancet* 1989; 1:1039-1041
7. Mao JS, Dong DX, Zhang HY, et al: Primary study of attenuated live hepatitis vaccine (H2 strain) in humans. *J Infect Dis* 1989; 159:621-624
8. Werzberger A, Mensch B, Kuter B, et al: A controlled trial of a formalin-inactivated hepatitis A vaccine in healthy children. *N Engl J Med* 1992; 327:453-457
9. Hollinger FB: Factors influencing the immune response to hepatitis B vaccine, booster dose guidelines, and vaccine protocol recommendations. *Am J Med* 1989; 87(suppl 3A):36S-40S